Supercritical Carbon Dioxide Fractionation of Crude Rice Bran Oil Using a Packed Column with Characterization of the Resultant Fractions

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Recently plant sterol (phytosterol) extracts have become important items of commerce due to their cholesterol-lowering properties. Phytosterols are common to many plant-derived oils, but represent only a very small fraction of the total oil composition. Previous studies have shown that rice bran oil and derivative products are an excellent source of nutritionally-beneficial ingredients, such as sterols, tocopherols, tocotrienols, and oryzanol (ferulate esters of plant sterols), all of which are claimed to have cholesterol-reducing properties. Unfortunately crude rice bran oil contains higher levels of free fatty acids than many other vegetable oils, due to the presence of high levels of an active lipase which promotes hydrolysis of oil to the free fatty acids. Conventional refining processes to remove the free fatty acids can result in a significant reduction of active rice bran oil components, consequently a high pressure fractionation process employing supercritical carbon dioxide was examined as an alternative process for reducing the free fatty acid content and concentrating the above components. An eight-foot column packed with Propak internals and operating semi-continuously, was used to remove free fatty acids from the crude oil. Crude rice bran oil, which was commercially extracted with hexane, was used as a feed material. Compositions of the extract and raffinate fractions from the column were analyzed for steryl esters, free sterols, and oryzanol content, as well as total triglyceride and free fatty acid content. The effect of pressure (13.6-34.0 MPa), both isothermal and temperature gradient (45-90°C) operation of the column, carbon dioxide flow rate and fractionation time on the composition of fractions were examined. The phytosterol content of a product from SC-CO_2 fractionation column was compared with a refined rice bran oil and phytosterol-enriched margarine. It was concluded that the SC-CO_2-based fractionation could be a viable technique for refining crude rice bran oil without the significant loss of oryzanol and triglyceride content during the processing.

INTRODUCTION

Phytosterols are minor components of all vegetable oils and fats. The cholesterol-lowering effect of such sterols has been reported in literature[1,2]. Rice bran oil is an excellent source of nutritionally-beneficial compounds, such as sterols, tocopherols and tocotrienols[3]. Rice bran oil's unsaponifiable fraction contains about 43% phytosterols, 10% steryl esters and 1% tocopherol[4]. The free fatty acid (FFA) content of crude rice bran oil is higher than that of many

other vegetable oils, due to the presence of high levels of an active lipase, which promotes hydrolysis of oil to FFA. Rice bran oil refining losses range between 18 to 22% during the conventional oil processing[4]. Furthermore, conventional refining processes to remove FFA can result in a significant reduction (~50%) of active rice bran oil components [5].

High-pressure extraction and fractionation technology employing supercritical carbon dioxide (SC-CO_2) is an alternative technique for oil extraction and refining. Several studies have reported SC-CO_2 extraction of rice bran oil. Taniguchi et al.[6] reported the presence of oryzanol in the SC-CO_2 extracts. Zhao et al.[7] showed that rice bran oil fractions obtained at higher pressures contained less FFA, waxes and unsponifiables. According to the Ramsay et al.[8] study total sterol content of the SC-CO_2-extracted rice bran oil was less than that of the hexane extracted oil. Shen et al.[9,10] investigated the pilot scale SC-CO_2 extraction of rice bran oil and calculated the solubility of rice bran oil, partition coefficients and selectivities of oil components as a function of the fluid temperature, pressure and density. Kuk and Dowd[11] extracted rice bran oil using SC-CO_2 and showed that the level of sterols in SC-CO_2 extracted oil increased with pressure and temperature.

Although lampante olive oil refining and deacidification of roasted peanut and olive oil[12,13,14] with SC-CO_2 have been studied before, the deacidication of crude rice bran oil using a SC-CO_2 fractionation (SFF) tower has not been reported up to date. Therefore, the objectives of this study were to deacidify commercially extracted crude rice bran oil using a SFF tower and to determine the optimal conditions for maximum FFA removal while minimizing the phytosterol and triglyceride (TG) losses during the process.

EXPERIMENTAL

Materials and methods

Crude rice bran oil, which was used as feed material for this study was received from Riceland Foods Inc. (Stuttgart, Arkansas). The crude oil was centrifuged at 3000 rpm for 20 minutes and the resultant precipitate was separated from the oil prior to the SFF experiments.

Triglyceride, FFA and steryl fatty acid ester (StE) contents of the samples were analyzed by HPLC according to Moreau et al.[15]. Details of the method are given by Dunford and King[16]. Free sterol and oryzanol compositions of the samples were determined by supercritical fluid chromatography (SFC) as reported by Dunford and King[16]. Triglyceride and FFA compositions

of the samples were reported as HPLC area percentages, whereas oryzanol, StE and free sterols were given as weight percentages unless otherwise stated. Each sample was injected at least twice and the averages were reported.

Column fractionation

The SFF experiments were carried out in a pilot-scale column. The total height and volume of the column were 164 cm and 260 cm^3, respectively. The column was packed with protruded stainless steel material (0.16 in Pro-Pak, Scientific Development Company, State College, PA), which provided 94% void volume in the column. A detailed description of the column design, temperature and pressure control systems was given by King et al.[17] and Dunford and King[16]. The column was operated in the semi-continuous mode; feed and CO_2 were in the batch and continuous mode, respectively. The duration of the fractionation experiments was 3 h unless otherwise stated. The CO_2 flow rate was 1.2 L/min as measured at room temperature and atmospheric pressure. Extract and raffinate samples were collected from the top and bottom of the column, respectively. The column was depressurized and residual oil was drained at the end of each run. Then the column was cleaned at 34.0 MPa and 90°C with flowing CO_2 for more than 6 h.

RESULTS AND DISCUSSION

Crude rice bran oil, which was used as feed material for this study contained 70±2 % TG, 7.1±0.5 % FFA, 1.3±0.1 oryzanol, 0.33 ±0.03 % free sterol and 3.6 ±0.3 % StE.

Triglycerides

Triglyceride content of the extracts decreased from 52.5 % to 36.5 % with increasing temperature from 45°C to 80°C at 20.5 MPa, respectively. This was due to the higher solubility of FFA in SC-CO_2, which resulted in greater selectivity for the FFA. Similar results have been reported for the SC-CO_2 deacidification studies performed with the other vegetable oils[12,13]. TG content of the extracts was in the range of 56-57 % at 34.0 MPa. TG content of raffinate samples (Fig. 1) was higher (72.2-76.3%) than that of the extract samples (36.3-56.6 %) and feed material (~70 %).

Free Fatty Acids

Free fatty acid content of the extracts collected at 34.0 MPa was lower than that of the lower pressure (Fig. 2). This was due to the higher SC-CO_2 selectivity for TG at higher pressures. The extract with the highest FFA concentration (36.6 %) was obtained at 20.5 MPa and 80°C. Free fatty acid content of the raffinate samples (5.1-2.9 %) was significantly lower than that of the extract samples (17.6-36.8 %).

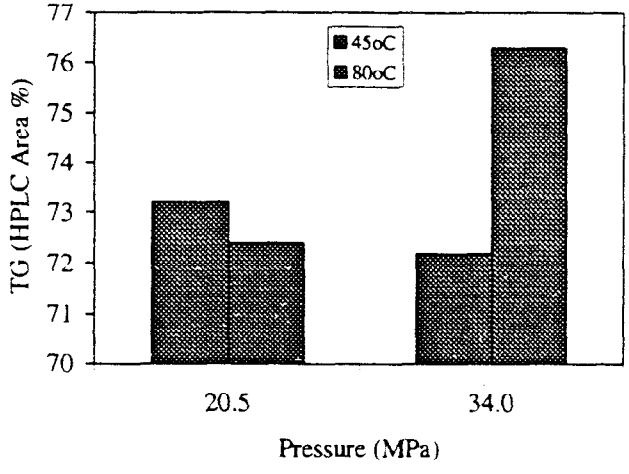

Figure 1: Effect of temperature and pressure on the TG content of raffinate samples.

Free Sterols

Free sterol content of the extract samples (Table 1) was higher than the raffinate samples (0.23-0.35 %) at all pressure and temperature conditions studied. However, sterol content of raffinate fractions was still similar to that of the feed material (0.33 %).

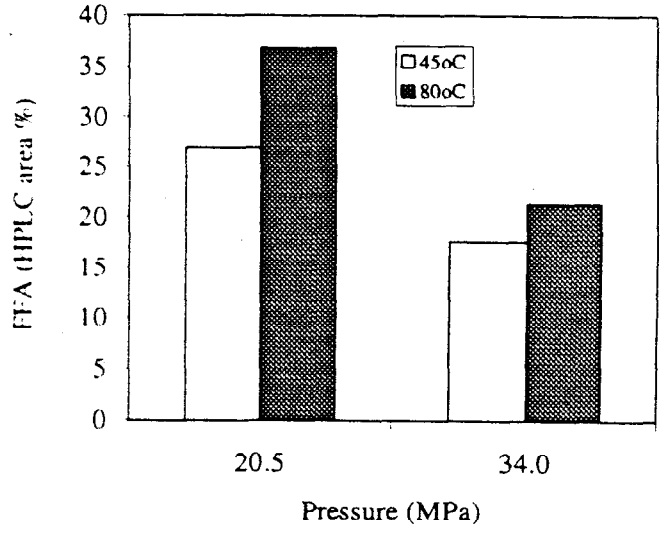

Figure 2: Effect of temperature and pressure on the FFA content of extract samples

Table 1: Effect of pressure and temperature on the phytosterol content of the fractions

Pressure (MPa)	**20.5**		**34.0**	
Temperature (°C)	**45**	**80**	**45**	**80**
Free sterol (Extract)	0.76	0.70	0.47	0.69
Oryzanol (Raffinate)	1.35	1.29	1.58	1.47
StE (Extract)	1.65	2.79	3.27	1.77

Oryzanol

Oryzanol content of the raffinate samples (Table 1) was significantly higher than that of the extract fractions (0.22-0.47 %). The implication of this finding is quite important for the application of SFF technology to rice bran oil deacidification, because rice bran oil, which is refined using conventional processes does not contain significant amount of oryzanol (Table 2).

Table 2: Lipid and phytosterol composition of a sterol-enriched margarine and rice bran oil samples deacidified using different processes. Oil compositions were expressed as SFC area percentages.

	TG	**FFA**	**Free Sterol**	**Oryzanol**	**StE**
Regular rice bran oil[1]	92.0	0.07	0.20	n.d.[2]	4.0
High oryzanol rice bran oil[3]	90.0	0.05	0.22	0.6	5.0
SFF processed rice bran oil[4]	92.0	0.08	0.35	1.8	3.5
Phytosterol-enriched margarine[5]	69.0	0.27	0.32	n.d.[2]	29.0

[1] Commercially refined using conventional caustic refining

[2]not detected

[3] Commercially refined using special techniques, which were not revealed by the processor

[4] The raffinate fraction from a SFF experiment, which was carried out at 13.6 MPa , 45°C and 1.2 L/min CO_2 flow rate

[5]Commercially available margarine

Sterol Fatty Acid Esters

Steryl fatty acid ester content of the raffinate samples (4.18-3.25 %) was higher than that of the extract samples (Table 1). SFF-deacidified products had similar StE content to the feed material.

Thermal gradient column operation

TG composition of the extract fraction collected during the thermal gradient column operation was lower than that of the isothermal operation (Table 3). This is due to the internal reflux created by the higher temperatures at the higher sections of the column. An increase in temperature at constant density results in lower solvent density and a decrease in TG solubility in the SC-CO_2 phase, consequently TG's stay in the column lowering the TG content of the extract leaving the column.

Table 3: Effect of isothermal and thermal gradient column operations on the extract composition.

	TG	FFA	Free Sterol	Oryzanol	StE
Isothermal[1]	52.5	26.9	0.76	0.24	1.65
Thermal Gradient[2]	45.7	29.8	0.9	0.22	2.35

[1]20.5 MPa, 45°C isothermal column operation, 1.2 L/min CO_2 flow rate and 3 h fractionation

[2]20.5 MPa, 45-45-55-65-75°C thermal gradient column operation, 1.2 L/min CO_2 flow rate and 3 h fractionation

CO_2 flow rate

Increasing CO_2 flow rate from 1.2 L/min to 2 L/min did not affect the composition of the extract fraction significantly (Table 4).

Table 4: Effect of CO_2 flow rate on the extract composition.

	TG	FFA	Free Sterol	Oryzanol	StE
1.2 L/min[1]	52.5	26.9	0.76	0.24	1.65
2.0 L/min[1]	49.7	27.6	0.81	0.20	2.07

[1] Crude rice bran oil fractionation was carried out at 20.5 MPa and 45°C.

Fractionation time

TG and FFA content of the extract samples changed significantly with the fractionation time (Table 5). TG content of the extract fractions decreased from 65.3 % to 10.6 % while FFA content of the same samples increased from 5.1 % to 59.3 % during a 480 min fractionation run. Although free sterol and StE content of the fractions was not affected significantly by the fractionation time, a slight decrease was observed in the oryzanol content of the extracts.

Table 5: Effect of fractionation time on the extract composition. Fractionation of the crude rice bran oil was carried out at 13.6 MPa and 45-45-60-80-90°C and 1.2 L/min CO_2 flow rate.

Fractionation time (min)	TG	FFA	Free Sterol	Oryzanol	StE
0-120	65.3	5.1	0.1	0.30	1.5
120-240	37.6	30.3	0.1	0.06	0.8
240-360	17.4	54.3	0.1	0.10	1.1
360-480	10.6	59.3	0.07	n.d.[1]	0.6

[1]not detected

Conclusion

In this study, we have shown that crude rice bran oil can be deacidified utilizing SFF technology without impairing the phytosterol content of the oil. Low pressure and high temperature processing is favorable for the crude oil deacidification since under these conditions phytosterol and TG losses with the extract fraction are lower and FFA concentration of the extracts are higher. In Table 4 lipid and phytosterol composition of three rice bran oil samples, which were processed using different refining processes, was given. The phytosterol content, particularly oryzanol content of the rice bran oil deacidified using SC-CO_2 was ~3X higher than the commercially available high oryzanol rice bran oil. These results clearly indicate that SFF can be an alternative process for crude oil deacidification and production of phytosterols enriched from vegetable oils.

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